Absorption of food colorant chlorophyll

Chlorophylls are commonly used food colorants. Epidemiological studies have shown correlation between the consumption of chlorophylls and the decreased risk of colon cancer. This model study with dogs evidenced a reduced absorption, and consequently, the maintenance of high amounts of chlorophyll derivatives in the digestive tract, agrees with the proposition that these pigments may exert a local activity in the gut, concerning chemopreventive properties. Recent studies have shown that chlorophyll derivatives can exhibit health-promoting activities, apart from their use as food and pharmaceutical colorants. Nevertheless, information regarding their absorption is almost inexistent. Therefore, the scientists at Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, Brazil calculated the apparent absorption of chlorophyll content in food in dogs. The eventual appearance of any chlorophyll derivative in the bloodstream was also investigated. Spinach was added to a commercial dog food and animals in the experimental group were held on this diet for 10 days. Chlorophylls a and b were transformed into their respective pheophytins during the G.I. passage. Beyond pheophytinization no other important degradation occurred. The apparent absorption of the chlorophyll derivatives ranged from 2.5 to 4.0%, with an average of 3.4%. In a second experiment, where dogs consumed a diet containing 10% dried spinach, no chlorophyll derivatives could be found in the peripheral blood until 150min after consumption, which seems to evidence either their low absorption or their quick metabolization. These results deserve further investigations to get a better understanding about the role of green vegetables in the human diet [Fernandes Taís Motta, Gomes Bárbara Bicalho and Lanfer-Marquez Ursula Maria, Apparent absorption of chlorophyll from spinach in an assay with dogs, Innov Food Sci Emerg Technol, 2007, 8(3), 426-432].

Fresh coffee husks as potential source of anthocyanins

Among the natural colorants anthocyanins are that range from yellow to bright red to blue. These pigments are water soluble and are currently being associated with health benefits, such as antioxidant capacity, anti-inflammatory properties and others. Brazil is the largest coffee producer in the world where processing of coffee generates expressive amounts of agricultural waste. Coffee husks (CH), comprised of outer skin, pulp and parchment, are probably the major residues from the handling and processing of coffee. Considering the utilization of such a useful waste the researchers of Brazil evaluated the potential of fresh coffee husks as source of anthocyanins. The extracted pigments were analysed by HPLC with photodiode array detection. Partial hydrolysis and analysis of the resulting fragments were helpful in the characterization procedure. Cyanidin 3-rutinoside was characterized as the dominant anthocyanin in fresh coffee husks and its quantification suggested the fresh coffee husks to be a good candidate as source of this pigment [Prata Emille RBA and Leandro S, Fresh coffee husks as potential sources of anthocyanins, JWT-Food Sci Technol, 2007, 40(9), 1555-1560].

Anthocyanins from Eugenia myrtifolia Sims

Eugenia myrtifolia Sims fruits have been characterized for their anthocyanin content and quality by researchers in Italy. Following the common procedures and validated methods for separation, identification and quantification of anthocyanins, the presence of malvidin 3,5-0-diglucoside as a unique anthocyanin (32.59mg/100g fresh weight of the fruit) was reported. An in vitro shoot culture of E. myrtifolia was established in order to explore the possibility to produce pigments in vitro. The presence of the only malvidin 3,5-O-diglucoside could be a useful feature in order to manipulate biotechnologically the anthocyanin biosynthetic pathway in the in vitro material (callus and suspension cultures) [Longo Luigia, Scardino Anna, Vasapollo Giuseppe and Blando Federica, Anthocyanins from Eugenia myrtifolia Sims, Innov Food Sci Emerg Technol, 2007, 8(3), 329-332].
Production of alizarin extracts from *Rubia tinctorum* Linn. and assessment of their dyeing properties

Madder (*Rubia tinctorum* Linn.) has been cultivated as a source of dyestuff since antiquity in central Asia and Egypt. It is a perennial plant which produces a red dye (alizarin) from its roots. In a study conducted by researchers at Dipartimento di Scienze e Tecnologie Agroalimentari, Università della Tuscia, Viterbo, Italy several experiments were performed in 50 cm³ shaken-tubes, thus allowing methanol to be selected as the most appropriate leaching solvent for alizarin from roots of *R. tinctorum*. Methanol at 25°C was found to be able to extract not only free alizarin but also its glycosidic forms, thus resulting in an overall alizarin extraction yield of 2.9±0.1 g/kg of dried material when leaching madder root particles with 100 dm³ of methanol/kg.

Further extraction tests using a liquid-solid ratio of 40 dm³/kg in an 1-dm³ stirred extractor allowed the production of a methanolic extract, which was then dried under vacuum. The solid residues were re-dissolved in ethanol so as to avoid methanol vapours exhaling from dyeing baths.

Dyed standard specimens of raw cotton and wool exhibited almost the same reddish-yellow hue, even if those coloured with the ethanolic extract had a lighter colour intensity and a more pinkish shade than those dyed with madder root particles. Whatever the dyeing procedure used, the colour intensity or hue of cotton specimens was found to be brighter or more pinkish than the wool ones. These characteristics were also more evident for the cotton specimens dyed with the ethanolic extract. The fastness properties of dyed cotton and wool specimens were evaluated and it was found that all the dyed specimens were not or just slightly affected by manual washing at 40°C, acid or basic perspiration tests and it was also found that the resistance to fading of dyed wool specimens was generally greater than that of cotton ones. Thus, it might be concluded that the colour components extracted by methanol from the roots of *R. tinctorum* would help to standardise the natural-dyeing procedures within statistically insignificant differences in colour intensity. At the same time, the textile manufacturer would not be burdened with the disposal problems of wet madder root residues. Thus, the dye extracted might be an alternative to synthetic dye for dyeing cotton and wool.

Dyeing of wool with natural anthraquinone dyes from *Fusarium oxysporum*

Researchers are currently investigating the production and evaluation of microbial pigments as textile colorants. Fungi are more ecological interesting source of pigments, since some fungal species are rich in stable colorants, such as anthraquinone. Two anthraquinone compounds are described by researchers at National Research Center, Dokki, Cairo, Egypt which were produced by liquid cultures of *Fusarium oxysporum* (isolate no. 4), isolated from the roots of citrus trees affected with root rot disease. These anthraquinone compounds are 2-acetyl-3,8-dihydroxy-6-methoxy anthraquinone or 3-acetyl-2,8-dihydroxy-6-methoxy anthraquinone. Dyeing of wool fabrics with these new anthraquinone compounds as natural dyes has been studied. The values of dyeing rate constant, half-time of dyeing and standard affinity have been calculated and discussed. The effect of dye bath pH, salt concentration, dyeing time and temperature were studied. Colour strength values and the dye uptake were high. The results of fastness properties of the dyed fabric were good. Thus, anthraquinone compounds which were produced by stationary cultures of *F. oxysporum* could be used for dyeing wool with good fastness properties and high dye uptake. They can serve as a noteworthy source of raw material in the future. [Nagia FA and EL-Mohamedy RSR, Dyeing of wool with natural anthraquinone dyes from *Fusarium oxysporum*, *Dyes Pigments*, 2007, *75* (3), 550-555].
Isolation of carotenoid pigments from red yeast

Carotenoids are of great commercial interest. They are used as natural food colorants and as a pigment source in fish diets. The red yeast *Rhodotorula glutinis* produces valuable carotenoid pigments which are used as natural food colorants and as a pigment source in fish diets. The chemists at Department of Chemical Engineering, University of Seoul, Republic of Korea and Department of Chemical and Process Engineering, University of Canterbury, New Zealand worked jointly; they cultivated *R. glutinis* and harvested for carotenoid extraction after 3 days of fermentation. Five different solvents were used to disrupt the cell wall of freeze-dried *R. glutinis* cells and thus release the intracellularly produced carotenoids. Chromatographic analysis showed that three carotenoid pigments (β-carotene, torulene and torularhodin) were present in the extract. A Box-Behnken experimental design was employed to investigate statistically the main and interactive effects of three of the five solvents tested (dimethyl sulfoxide, petroleum ether and acetone) and saturated NaCl solution on the extent of extraction. An empirical model relating extraction yield to the four variables was developed on the basis of the experimental results. The model was shown to be a statistically significant description of the carotenoid extraction process and could be successfully used to derive the most efficient combination of the four variables to extract carotenoids from *R. glutinis*. Extraction conducted under the optimum conditions resulted in a high yield of carotenoids, suggesting that the combined chemical cocktail was effective at compromising the integrity of the *R. glutinis* cell wall [Park PK, Kim EY and Chu KH, Chemical disruption of yeast cells for the isolation of carotenoid pigments, *Separat Purific Technol*, 2007, 53(2), 148-152].

Isolation of chlorophylls from stinging nettle

Young leaves of stinging nettle (*Urtica dioica* Linn.) are cooked as a potherb, added to soups and can also be dried for winter use. Nettle is a very nutritious food that is easily digested and is high in minerals (especially iron), vitamin C and pro-vitamin A. The researchers at Faculty of Chemistry and Chemical Engineering, University of Maribor, Slovenia assessed the feasibility of extracting chlorophylls from stinging nettle by solvent extraction. At preliminary stage three parts of raw material (leaves, stalk and whole plant), different preservation techniques (blanching and drying) and different storage temperatures of preserved material (-20°C for blanched and 4°C for dried sample) were taken into consideration. Extracts were analysed for chlorophyll A and B, respectively, by means of HPLC method. Afterwards, optimization of single-step conventional extraction was carried out by examining the influence of the following process parameters on the extraction yield and isolation efficiency of chlorophylls: type of organic solvent, content of water in ethanol/water mixtures, ratio solvent/material, temperature, time of extraction and pH of the extraction mixture. Furthermore, to improve the yield and/or the selectivity of the extraction, multi-step conventional extraction experiments as well as other solid-liquid extraction techniques (soxhlet and ultra-sound assisted) were performed and compared. The results showed that much higher concentration of chlorophylls in extract as well as higher yields and isolation efficiencies were obtained in this way. Finally, various extraction techniques were performed and examined, and they showed that ultra-sound assisted extraction is a promising alternative extraction technique giving the highest efficiency of extraction [Maša Hojinik, Moja Škerget and Zeljko Knez, Isolation of chlorophylls from stinging nettle (*Urtica dioica* L.), *Separat Purific Technol*, 2007, 57(1), 37-46].
Microwave-assisted rapid extraction of red dye from sappan heartwood

Sappan, *Caesalpinia sappan* Linn., heartwood dye has been well-known for its medicinal and dyeing properties. The researchers at Department of Pharmaceutical Chemistry, J. S. S. College of Pharmacy, Ootacamund, Tamil Nadu, India, isolated red dye using both conventional and newly developed microwave method. The conventional heating of 2h provided 0.656 +/- 0.049g of the dye and by microwave heating at 540W for 20 min, the yield obtained was 0.747 +/- 0.047g. Both the dyes were found to be the same as evidenced by UV, TLC and HPTLC studies. Antioxidant activity of the dyes was also carried out using DPPH and nitric oxide methods to confirm the similarity in their biological activity. The procedure developed can be used for the fast extraction of the red dye of *C. sappan* without affecting the nature of the product [Badami S, Geetha B, Sharma SV, Rajan S and Suresh B, Microwave-assisted rapid extraction of red dye from *Caesalpinia sappan* heartwood, *Nat Prod Res*, 2007, 21(12), 1091-1098].

Colour stability improvement of strawberry beverage by fortification with polyphenolic copigments of rose petals

Anthocyanin pigments are not only important defining the aesthetic value of foods and beverages, but also play a significant role from a nutritional point of view. The attractive red colour is one of the visual quality attributes strongly affecting consumer acceptance both of fresh and processed strawberry fruits. Unfortunately, due to the low total content of strawberry anthocyanins and their inherent heat and light sensitivity an accelerated pigment degradation occurs during conventional processing and storage, and retention of strawberry colour has always been a technological challenge.

Recently, industrially distilled petals of *Rosa damascena* Mill. were established as a rich source of flavonols, which have been demonstrated as highly effective polyphenolic copigments at low copigment/pigment ratio. Therefore, a study was conducted at University of Food Technologies, Plovdiv, Bulgaria to evaluate the copigmentation behaviour and heat stability of purified strawberry anthocyanins in the presence of rose petal polyphenolics. Additionally, the colour changes of strawberry beverage were monitored during thermal treatment when polyphenolic copigments naturally occurring in rose petals were added.

The anthocyanin degradation ideally followed first-order reaction kinetics ($R=0.99$) and the half-life value increased significantly due to the addition of rose petal polyphenolics. Colour stability increased due to the addition of rose petal polyphenolics, as the total colour difference was smaller for the fortified beverage, especially after prolonged heating (4h). The results obtained demonstrated that the addition of polyphenolic copigments extracted from distilled rose petals reduces the thermal degradation of strawberry anthocyanins, allowing improved colour stability of the processed strawberries. Moreover, this polyphenolic fortification could be worthwhile not only from technological point of view, but also with respect to the development of functional foods and beverages [Mollov Plamen, Mihalev Kiril, Shikov Vasil, Yoncheva Nikolina and Karagozov Vasil, Colour stability improvement of strawberry beverage by fortification with polyphenolic copigments naturally occurring in rose petals, *Innov Food Sci Emerg Technol*, 2007, 8 (3), 318-321].
Dyeing of wool and silk with Annatto

Annatto (Bixa orellana Linn.) is a small tree found in tropical countries of the world. Colouring matter is obtained from this tree when pulpy portion of its seeds is macerated. Annatto is commonly used as edible colour in butter, margarine, cheese, dairy products, biscuits and chocolates. Researchers in India aimed their study at applying such colorant on wool and silk fabrics and assessing some fundamental parameters related to dyeing of these fibres using such colorants.

Silk and wool fabrics have been dyed employing extract of seeds of annatto in absence and presence of magnesium sulphate, aluminium sulphate and ferrous sulphate. Colouration of both the fibres is found to be most effectively accomplished at pH 4.5 commonly in the absence and presence of such inorganic salts. Colour uptake for wool is found to be more than that for silk under all the conditions studied. The use of ferrous sulphate produces significant improvement in colour uptake when both the substrates are treated with such salt prior to application of annatto. Coloured protein fibres, in general, produce light and wash fastness ratings of 2-3. Ferrous sulphate, however, improves colour fastness properties and colour retention on washing of wool and silk fibres [Das Debasis, Maulik Sankar Ray and Bhattacharya Subhash Chandra, Dyeing of wool and silk with Bixa orellana, Indian J Fibre Text Res, 2007, 32 (3), 366-372].

Syzygium cumini Skeels fruit peel ó
A potential source of natural colorant

Indian black plum or Java plum is a tropical edible fruit obtained from the trees of Syzygium cumini Skeels syn. Eugenia jambolana Lam.; E. cumini Druce (Hindi — Jamun). The fruits are oblong berries, deep purple or bluish in colour with pinkish pulp, having various medicinal properties and used in Ayurveda as a stomachic, astringent, antiscorbutic, diuretic, antidiabetic, and in chronic diarrhoea and enlargement of spleen. The deep purple colour of the fruit is due to anthocyanins, in addition to their colourful characteristics, anthocyanins are known to possess excellent antioxidant properties. The low stability of anthocyanins at high temperature and light conditions is a limiting factor in their application as colorants.

Therefore, in a study conducted by scientists at Central Food Technological Research Institute, Mysore, India, anthocyanin pigments from S. cumini fruit peels were characterized and evaluated for their antioxidant efficacy and stability as extract and in formulation. Total anthocyanin content was 216mg/100ml of extract which is equivalent to 230mg/100g fruit on a dry weight basis. Three anthocyanins were identified as glucoglucosides of delphinidin, petunidin and malvidin by HPLC–ESI–MS. The antioxidant capacity of the extract was tested using models, such as scavenging, reducing power assay, lipid peroxidation in rat brain, liver, liver mitochondria, testes and human erythrocyte ghosts. The extract showed 78.2% DPPH-scavenging at 2.5ppm, while BHA exhibited only 41.6% activity at the same concentration, thus proving it to be a more efficient free radical-scavenger than the widely used BHA. One ppm of the extract was equivalent to 3.5µM ascorbic acid, as estimated by reducing power assay. Inhibition of rat brain lipid peroxidation was 94.4% at 5.0ppm concentration. It was almost equally active in all the biological models, except human erythrocyte ghost cells, where it showed only 48% inhibition at 5.0ppm. The extract was quite stable at 0°C with 11% loss in 4 weeks, while the pigment loss in the antitussive formulation was only 13% at 30°C at the end of 8 weeks. The high antioxidant activity and relatively high stability of the pigments make S. cumini a potential source of natural colorant as well as antioxidants. The anthocyanins, like all the other anthocyanins, have the advantage of high solubility in aqueous mixtures, imparting an attractive colour that makes their incorporation easy into numerous aqueous food and non-food formulations, including pharmaceuticals. The extract is a rich source of anthocyanins whose content is equivalent to that of blue berries and black currants and higher
In recent years, the use of low-environmental impact biotechnology is giving new types of treatment in the textile industry. The use of protease enzymes to improve some physical and mechanical properties such as smoothness, drapeability, dyeing affinity and water absorbency is particularly interesting. In a study carried out at Color Control and Color Reproduction Department, Institute for Colorants, Paint and Coating, Tehran, Iran, wool yarns were first treated with different concentrations of protease enzymes in water solution including 1, 2, 4 and 6% o.w.f. (on weight of fabric) for 1h. The dyeing process was then carried out on the treated yarns with madder (50% o.w.f.). Tensile strength of treated yarns was decreased due to enzyme treatment and it continued to decrease with an increase in enzyme concentration in solution. The $L^*$ values decreased for the samples treated with enzyme. The wash and light fastness properties of samples were measured according to ISO 105-C05 and Daylight ISO 105-BO1. The washing fastness properties of treated samples were not changed. In the case of light fastness properties, it was increased a little for 4 and 6% enzyme treated samples. Scouring process disrupts hydrophobic barrier at the fibre surface where it allows the proteases to reach the protein layers of the wool cuticle as a whole. Protease damages the cuticle’s edge and overlapping area and to progressive damage to the matrix proteins and to the macrofibrils in the fibre cortex, allowing the dye molecules to penetrate more easily into the fibre cortex [Parvinzadeh Mazeyar, Effect of proteolytic enzyme on dyeing of wool with madder, Enzyme Microb Technol, 2007, 40 (7), 1719-1722].

**Effect of proteolytic enzyme on dyeing of wool with madder**

than that of blackberries, all widely acclaimed anthocyanin-rich edible fruits. Like grape anthocyanins that are sold commercially as oenocyanin, the peel powder of *S. cumini* may also be employed as a colorant for foods and pharmaceuticals [Veigas Jyothi M, Narayan Mandayam S, Laxman Padmere M and Neelwarne Bhagyalakshmi, Chemical nature, stability and bioefficacies of anthocyanins from fruit peel of *Syzygium cumini* Skeels, Food Chem, 2007, 105 (2), 619-627].

**Feed/Fodder**

Response of laying Japanese quails to graded levels of essential amino acids

The effect on egg production of graded levels of ideal amino acids, combined with reduced protein in the diet, was investigated by the scientists of Central Avian Research Institute, Izzatnagar and Rohilkhand University, Bareilly, Uttar Pradesh, India. During experiment 312 laying quails aged 6-18 weeks were offered six diets, each of which contained one of three levels of amino acids [85, 100 and 115% of essential amino acids (EAAs)] together with 5% or without fishmeal (FM) (3 × 2 factorial design). Each diet was offered to 26 replicated groups of two quails each. Hen-day and hen-housed egg production did not differ as a result of EAA level, protein type or their interaction during the overall period of egg production. Egg weight improved linearly ($P<0.01$) with increased EAA levels, while egg mass output per bird per day remained similar at the 100 and 115% EAA levels. Quails fed higher (100 and 115%) EAA levels had an improved feed conversion ratio ($P<0.01$) compared to birds fed 85% EAA. The gain in body weight during the laying period was higher ($P<0.01$) at the 100 or 115% than at 85% EAA levels. The ratio of egg mass or egg mass and live weight gain, together, to protein intake improved ($P<0.01$) linearly with a decrease in EAA levels in the diets, while better ($P<0.01$) energy efficiency (EE, energy intake: egg mass) and net EE (energy intake: egg mass plus gain) was obtained in higher EAA levels (100 or